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Therapeutic management of Babesiosis alone and its mixed infection with Theileriosis in Cattle

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Abstract

Babesiosis being economically important haemoprotozoan infection of cattle, the present study was planned to evaluate the therapeutic efficacy of imidocarb dipropionate in babesiosis in cattle. The animals admitted to College clinics with a history of red urine, anemia, and jaundice were screened for babesiosis by using Giemsa stained blood smears. A total of 18 cattle found positive for *Babesia spp.,* out of which 7 had only Babesia infection while 11 were positive for both *Babesia* and *Theileria spp*. Prominent clinical signs were fever, anorexia, depression, rapid respiration, tachycardia, pale to icteric mucous membranes, coffee-colored urine, straining and normal defecation. Haematology showed a significant reduction in haemoglobin, total erythrocyte count, packed cell volume while a significant decrease in lymphocyte and neutrophil count. Cattle affected with *Babesia spp.* were treated with imidocarb dipropionate @ 1.2 mg/kg SC once while cattle suffering from a mixed infection of *Babesia spp.* and *Theileria spp.* were treated with a single dose of each imidocarb dipropionate @ 1.2 mg/kg SC and buparvaquone @ 2.5 mg/kg IM. Common supportive therapy of Inj. D25% @ 1 lit iv, Inj. Meloxicam+Paracetamol @ 0.5 mg/kg IM single dose, wherever required, Inj. B_1+B_2+B_6 @ 10 ml IM daily for seven days and Ferrous fumerate + Vit B_{12} + Folic acid @ one bolus PO BID daily for 20 days was used in all study cattle. Treated cattle showed clinical recovery of 85.72% in Babesiosis alone while 72.73% recovery in mixed Babesiosis and Theileriosis infection.

Keywords: Babesiosis, Cattle, Imidocarb Dipropionate, Treatment

Haemoprotozoan diseases are widely prevalent in Indian livestock. Babesiosis occupies the second position among haemoprotozoan diseases occurring in cattle in India (Vahora et al., 2012). In general, *Babesia bigemina* and *Babesia bovis* are infections of the tropics and subtropics (Radostits et al., 2010). In India, *Babesia bigemina* is the main species prevalent in bovines (Ruprah, 1985). The disease is transmitted under natural conditions from affected to healthy animals through ticks *Riphicephalus microplus* and *Riphicephalus annulatus* (Murrell et al., 2001). The disease has been recognized as a severe problem of major economic importance in bovines, and the financial losses in India due to babesiosis are INR 580.16 crore annually (Narladkar, 2018). The economic losses occur in the form of death, decreased production, and lowered working efficiency of the affected animals in tropical and sub-tropical parts of the world, including India (Chakrabarti, 2016). Many animals die or undergo prolonged convalescence entailing the loss of milk production. Incidental costs of treatment add to the economic burden (Radostits et al., 2010). Indigenous cattle breeds from Babesia endemic regions often have a certain degree of natural resistance to these diseases, and the consequences of infection are not as serious as when exotic *Bos taurus* breeds are involved (Bock et al., 2004).

Diminazine aceturate and Imidocarb dipropionate are the drugs recommended for the treatment of babesiosis in livestock. Diminazine works rapidly against *B. bovis* and *B. bigemina* @ 3.5-7 mg/kg intramuscularly. It is well-tolerated and protects cattle from both Babesia infections for 2 and 4 weeks, respectively. Imidocarb dipropionate is also an effective antibabesial drug, which is used subcutaneously @ 1.2 mg/kg for treatment while @ 3 mg/kg provides protection from *B. bovis* for 4 weeks and *B. bigemina* for at least 2 months (Bock et al., 2004). It is the only babesiacide that consistently clears the host of parasites, and cattle treated with imidocarb may end up with a solid, sterile immunity (Lewis et al., 1981). It protects from clinical disease for 3 to 6 weeks and allows a sufficient level of infection for immunity to develop which is interesting in areas where babesiosis is endemic.

Diminazine diaceturate is the most widely used drug for the treatment of babesiosis in India as it is commercially available. However, in clinical practice, it has been observed that some cases of babesiosis are not responding to even two doses of Diminazine aceturate. Recently imidocarb dipropionate has been

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made commercially available in India. Therefore, the present study was aimed at evaluating the efficacy of imidocarb dipropionate in the treatment of babesiosis in cattle.

Materials and Methods

The present study was conducted at Teaching Veterinary Clinical Complex, College of Veterinary and Animal Science, Udgir, during the period from January 2018 to June 2019. All the suspected cases were subjected to recording history and detailed clinical examination upon admission. Vital parameters like body temperature, respiration, heart rate, mucous membrane color were examined in all cases. Similarly, observations on the act of urination, defecation, the color of urine, consistency of feces, and size of lymphnodes were noted. Evidence of tick infestation in ailing animals was also recorded.

About 2 ml of blood was collected by jugular venipuncture in EDTA vials, and complete blood count was carried out on an automated haematology analyzer (Diatron Abacus Junior Vet, 3.11, Austria).

Blood samples collected in EDTA vials were immediately processed for blood smear preparation. The blood smears prepared were fixed using absolute methanol and stained with Giemsa stain (1: 10 ratio) for 25 to 30 minutes.

Babesiosis in cattle was diagnosed based on clinical findings, haematology and blood smear examination as well as response to anti-protozoal drug imidocarb dipropionate.

All confirmed cases of *Babesia spp.*only were treated with a single dose of Imidocarb dipropionate @ 1.2 mg/kg (Inj. Babimido®)* by SC route while cattle suffering from mixed infection of *Babesia spp.* and *Theileria spp.* were treated with a single dose of Buparvaquone (Inj. Zubion)** @2.5 mg/kg IM along with Imidocarb dipropionate (Inj. Babimido) @ 1.2 mg/kg SC. In both groups before administration of Imidocarb dipropionate, atropine sulfate was administered @ 0.03 mg/kg IM for control of cholinergic signs. Supportive therapy for all study cattle comprised of D25 @ 1 lit slow IV for 3 days, Meloxicam + Paracetamol (Melonex plus®) @ 0.5 mg/kg IM single dose (if required), Inj. B_1+B_2+B_6 @ 10 ml IM daily for 7 days (Tribivet®) and Ferrous fumerate + Vit B_{12} + Folic acid @ 1 bolus PO BID daily for 20 days (Bol. Feritas®). The efficacy of treatment was adjudged with improvement in clinical and haematological parameters in treated cattle.

*Babimido- Brand of Zydus Animal Health, Ahmedabad
**Zubion, Tribivet, Feritas, Melnoex plus - Brand of Intas Pharmaceuticals, Ahmedabad

Statistical analysis

Data about various observations in diseased animals and healthy animals as well as before treatment and after recovery was analyzed by Paired‘t’ test for unequal and equal numbers of animals as per Snedecor and Cochran (1994).

Results and Discussion

Eighteen cattle, including twelve female cattle and six male cattle confirmed for babesiosis on blood smear examination, were included in the study. Out of 18 cattle, seven animals were indigenous, whereas 11 were crossbred cattle. Out of 12 female cattle ailing with babesiosis alone or mixed infection, 11 were in early lactation (< 45 days) while one cow was in late lactation (> 6 months). The finding of the highest incidence in crossbred cattle in early lactation is in agreement with the findings of Velusamy et al. (2014) and Radostits et al. (2010).

The common clinical signs observed in babesiosis affected cattle were fever, pale icteric to red icteric mucous membranes, haemoglobinuria, and straining during defecation. Similar findings were noted in babesiosis by numerous workers (Bhikane et al., 2001; Tufani et al., 2009; Shinde et al., 2019 and Abd El-Hamed et al., 2016). The variable degree of weakness, dullness and depression was observed in affected cattle, probably due to anemia. Coffee colored urine color in ailing animals might be attributed to increased intravascular haemolysis (Shinde et al., 2019).

The clinical signs recorded viz., fever, anemia, icterus, and sometimes death of affected animals may be attributed to the endogenous pyrogens liberated by haemoprotozoan parasites causing destruction of erythrocytes and triggering various haemopoietic and thermoregulatory centers of the body (Abd El-Hamed et al., 2016). In the present study, a highly significant ($P<0.01$) increase in body temperature, pulse rate, respiratory rate, and decrease in ruminal motility was observed in babesiosis affected cattle as compared to healthy animals (Table 1 and Fig 1-3).

The elevated body temperature, heart rate and

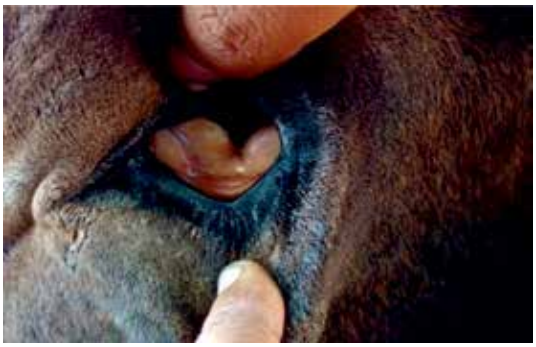

Fig 1: Icteric-red mucous membrane

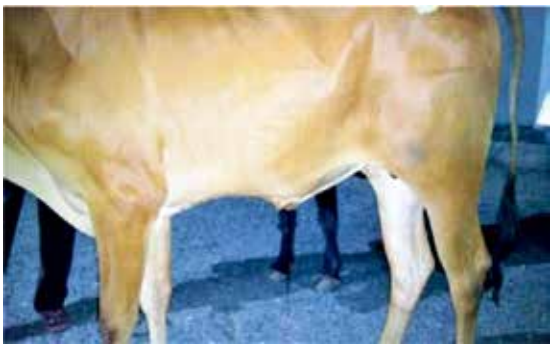

Fig 2: Coffee colored urine

Fig 3: Drowsiness, dull coat and recumbency

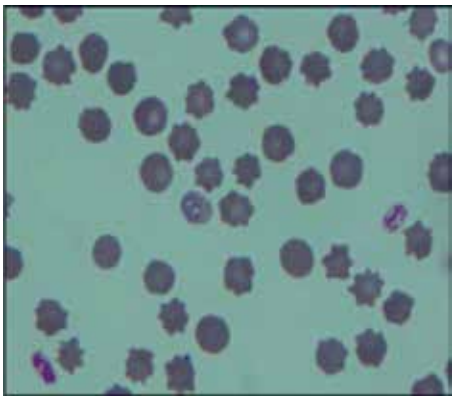

Fig. 4: Mild Babesia bigemina infection

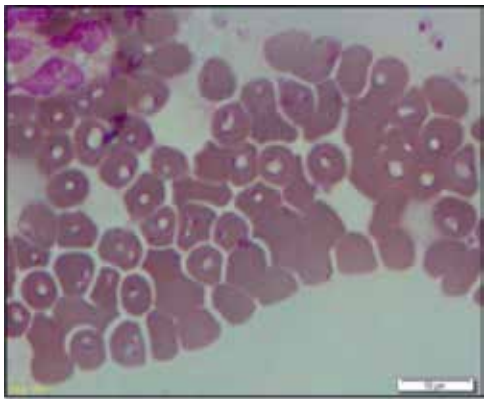

Fig. 5: Heavy Babesia spp. infection

respiration rate in affected cattle could be attributed to liberation of endogenous cytokines like tumor necrosis factor-α (TNF-α) and interleukins (IL-1, and IL-6) from infected mononuclear cells and thereby stimulation of thermoregulatory center in the hypothalamus in haemoprotozoan infections (Forsyth et al., 1999; Glass et al., 2003).

In the present study, the cattle affected with babesiosis showed highly significant (P<0.01) decrease in Hb, PCV and TEC (2.99 ± 0.35 *vs* $6.40 \pm 0.49 \times 10^6$/ µl) with non-significant changes in MCV and MCHC values as compared to healthy cattle (Table 1). The present findings on Hb, PCV, and TEC are in agreement with those reported by Bhikane et al. (2001), Wadhwa et al. (2008), and Shinde et al. (2019) in babesiosis-affected cattle. The significant (P<0.05) decrease in lymphocyte count and neutrophil count was observed in affected animals as compared to healthy ones (Table 1). Similar changes in leukogram were earlier noticed in babesiosis (Bhikane et al., 2001; Shinde et al., 2019; Abd El-Hamed et al., 2016) in affected cattle. The

Table 1: Mean physiological and haematological values in healthy, and babesiosis (with and without theileriosis) affected cattle

Sr. No.	Parameter	Babesiosis affected (n= 18)	Healthy cattle (n=8)	't' value
1	Body temperature (ºF)	103.12 ± 0.51	101.19 ± 0.18	3.439**
2	Pulse rate (Per minute)	82.00 ± 3.41	67.28 ± 3.00	3.229**
3	Respiration (Per minute)	33.89 ± 1.82	23.83 ± 1.28	4.497**
4	Rumen motility (per 5 min)	2.55±0.34	5.27±0.57	-4.060**
5	Hb (g/dl)	5.03 ± 0.43	9.76 ± 0.51	6.966**
6	PCV (%)	14.23 ± 1.30	28.45 ± 1.43	7.318**
7	TEC ($\times 10^6$ /µl)	2.99 ± 0.35	6.40 ± 0.49	5.572**
8	MCV (fl)	50.27 ± 2.19	45.62 ± 1.83	-1.647^{NS}
9	MCH (pg)	17.51 ± 0.79	15.49 ± 0.47	-2.240*
10	MCHC (g/dl)	35.27 ± 1.28	34.31 ± 0.42	-0.741^{NS}
11	TLC ($\times 10^3$ /µl)	7.73 ± 0.90	9.77 ± 0.51	1.403^{NS}
12	PLT (x 10^3/µl)	87.44 ± 16.15	224 ± 25.32	4.544**

*NS-Non significant *-Significant (p<0.05) **-Highly significant (p<0.01)*

decline in the values of Hb, TEC, and PCV noticed in present study was attributed to lysis of erythrocytes due to presence of intra-erythrocytic piroplasms, alteration in antigenicity of erythrocytes due to entry of parasites, leading to autoimmune reaction in the body triggering removal of infected erythrocytes from circulation (Abd El-Hamed et al., 2016; Tufani et al., 2009). The changes in TLC and DLC in infected animals might have resulted from the breakdown of red blood cells by *Babesia spp*. thereby causing stimulation of the phagocytic cells such as lymphocytes and monocytes to clean up the body from the toxic remnants of ruptured red blood cells, as well as increased tissue demand for neutrophils that reduce the neutrophils in the peripheral circulation (Abd El-Hamed et al., 2016).

Highly significant (P<0.01) decrease in platelet count was observed in babesiosis affected cattle compared to the healthy ones. Similar observations were earlier made by Radostits et al. (2010); Tufani et al. (2009) and Shinde et al. (2019).

Giemsa-stained blood smears have been considered the "gold standard" for detecting *Babesia* inside the blood in acute instances. Cattle were diagnosed for babesiosis on the basis of the presence of intra-erythrocytic piroplasms in forms such as pear-shaped and paired in *Babesia bigemina* infection in blood smears (Fig. 4-5). There was also the presence of marked acanthocytosis, poikilocytosis, along with the presence of ghost cells and marked polychromasia indicating intravascular hemolysis and regenerative anemia.

Microscopic examination of the blood smear is the most straightforward and most accessible diagnostic test for field veterinarians. During acute infections, microscopy is reasonably sensitive for detecting the intraerythrocytic piroplasms in Giemsa stained blood smears.

The diagnosis of babesiosis was made based on the evidence of tick infestation, persistent illness in spite of routine antibiotic therapy, icteric red mucous membranes and coffee-colored urine with elevated body temperature, low Hb, PCV and TEC values, positive Giemsa stained blood smears and good response to imidocarb dipropionate treatment.

In the present study out of 18 cases of haemoprotozoan infections in cattle, 7 cases of babesiosis were treated with single dose of imidocarb dipropionate @ 1.2 mg/kg SC, and 11 cases of concurrent babesiosis with theileriosis were treated with combination of imidocarb dipropionate @ 1.2 mg/kg SC and buparvaquone @ 2.5 mg/kg IM. The duration of illness in cases ranged between 2 to 7 days. Out of 18 cases treated, 14 recovered entirely with a recovery rate of 77.77% (Table 2). Four cattle, especially young calves, did not show response to treatment due to severe haemolytic anemia. Qayyum et al. (2010) reported 100% efficacy of Imidocarb dipropionate @ 1.2 mg/kg in babesiosis affected cattle while buparvaquone @

Table 2: Recovery rate in affected cattle treated for Babesiosis

Group	Haemoprotozoan infections	No treated	No recovered	Percent recovery
Group II	*Babesia spp.*	7	6	85.72
	Babesia spp. + Theileria spp.	11	8	72.73
	Total	18	14	77.77

2.5 mg/kg along with long-acting oxytetracycline was found effective in mixed infections with supportive treatment for anemia.

In treated cattle, urine color was restored to normal straw color in 24-48 hours after treatment (Fig. 6) owing to clearance of haemoprotozoan infection and thereby intravascular haemolysis. Treated cattle showed increased alertness, strength, and improvement in appetite and water intake by 3-5 days of treatment. The pale mucous membranes became pink, body condition improved, and skin coat regained usual luster by 21st day of treatment. Highly significant (P<0.01) decrease in body temperature (103.12 ± 0.51 *vs.* 101.19 ± 0.18ºF), pulse rate (82.00 ± 3.41 *vs.* 67.28 ± 3.00 per min), respiratory rate (33.89 ± 1.82 *vs.* 23.83 ± 1.28 per min) and increase in ruminal motility (2.55 ± 0.34 *vs.* 5.27 ± 0.57 Per 5 min) was observed in treated cattle after recovery (Table 3).

Significant (P<0.05) elevation in Hb (5.03 ± 0.43 *vs* 6.33 ± 0.45g/dl), PCV (14.23 ± 1.30 *vs* 18.08 ± 1.40%) and TEC values (2.99 ± 0.35 *vs* 4.52 ± 0.48 × 10^6/µl) with non-significant increase in TLC (7.73 ± 0.90 *vs* 9.10 ± 1.28 × 10^3/µl), PLT (87.44 ± 16.15 *vs* 145.43 ± 27.16 x 10^3/µl), MCV (50.27 ± 2.19 *vs* 46.43 ± 2.21 fl), MCH (17.51 ± 0.79 *vs* 15.75 ± 0.83pg) and MCHC (35.27 ± 1.28 *vs* 34.06 ± 1.27 g/dl) was observed in treated cattle compared to pre-treatment values. These changes may be attributed to response to standard haemoprotozoan treatment along with supportive administration of haematinics such B complex and iron preparations.

Babesiosis in cattle was successfully treated with Imidocarb dipropionate with 85.72% recovery rate, and mixed infection was treated with imidocarb and buparvaquone with 72.73% recovery rate. In the pilot study, signs of adverse reaction to imidocarb dipropionate such as salivation, nasal discharge, restlessness, and colic were observed in treated cattle. However, in the present study, no cholinergic signs were observed due to prophylactic use of atropine sulfate before administration of imidocarb dipropionate. In this way, the results of the present research signify the utility of Imidocarb dipropionate in the therapeutic management of Babesiosis in cattle.

Table 3: Mean physiological and haematological values before and after treatment in cattle affected with babesiosis

Sr. No.	Parameter	Before treatment (n=18)	After treatment (n=14)	't' value
1.	Body temperature (ºF)	103.12 ± 0.51	101.19 ± 0.18	3.439**
2.	Pulse rate (Per minute)	82.00 ± 3.41	67.28 ± 3.00	3.229**
3.	Respiration (Per minute)	33.89 ± 1.82	23.83 ± 1.28	4.497**
4.	Rumen motility (/5 min)	2.55±0.34	5.27±0.57	-4.060**
5.	Hb (g/dl)	5.03 ± 0.43	6.33 ± 0.45	-2.151*
6.	PCV (%)	14.23 ± 1.30	18.08 ± 1.40	-2.039*
7.	TEC (× 10^6/µl)	2.99 ± 0.35	4.52 ± 0.48	-2.651*
8.	MCV (fl)	50.27 ± 2.19	46.43 ± 2.21	1.220^{NS}
9.	MCH (pg)	17.51 ± 0.79	15.75 ± 0.83	1.491^{NS}
10.	MCHC (g/dl)	35.27 ± 1.28	34.06 ± 1.27	0.637^{NS}
11.	TLC (× 10^3/µl)	7.73 ± 0.90	9.10 ± 1.28	-0.909^{NS}
12.	PLT (x 10^3/µl)	87.44 ± 16.15	145.43 ± 27.16	-1.836^{NS}

***-Highly significant (p<0.01), *-Significant (p<0.05)*

Fig 6: Recovery of urine color within 24 hrs after treatment with imidocarb

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